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RESEARCH ARTICLE

Composition of the essential oil of Pink Chablis™ bluebeard (*Caryopteris ×clandonensis* 'Durio') and its biological activity against the yellow fever mosquito *Aedes aegypti*

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Abstract

Caryopteris ×clandonensis A. Simmonds ex C. H. Curtis 'Durio' Pink Chablis™, (Lamiaceae) a pink-flowered cultivar distinctive among the typically blue-flowered cultivars of bluebeard, is valued as a small, deciduous shrub in the landscape for its mounded growth habit, showy flower display in summer, and attractiveness to insect pollinators. As part of a broader research program examining aromatic compounds from ornamental species as natural alternatives to synthetic chemicals for control of insect pests, the essential oil of Pink Chablis™ bluebeard was investigated for its chemical composition and bioactivity as a repellent and larvicide against the yellow fever mosquito [*Aedes aegypti* (L.) (Diptera: Culicidae)]. Essential oil from the aerial parts of this mildly aromatic ornamental species was extracted by water distillation and analyzed by gas chromatography and gas chromatography mass spectrometry. The primary compounds in the essential oil were α-copaene (8.3%), limonene (7.2%), and δ-cadinene (6.3%), followed by *trans-p*-mentha-2,8-dien-1-ol (4.6%), *trans-p*-mentha-1(7),8-dien-2-ol (4.5%), *cis-p*-mentha-2,8-dien-1-ol (4.0%), and hotrienol (3.8%). Against the yellow fever mosquito, the essential oil exhibited mild repellency compared to DEET (*N*,*N*-diethyl-3-methylbenzamide) as a reference standard. It exhibited weak activity as a mosquito larvicide.

Keywords: *Caryopteris ×clandonensis*, *Aedes aegypti*, mosquito control, mosquito larvicide, mosquito repellent

Introduction

Aedes aegypti L. (Diptera: Culicidae), the yellow fever mosquito, transmits viral pathogens, including yellow fever, dengue fever, and chikungunya, which can cause serious human illness and death (World Health Organization, 2014a, 2014b, 2014c). Insecticides have been the primary control measure for mosquito management, as well as control of a wide range of other insect pests in agriculture and public health situations. Frequent use of any single insecticide class, such as pyrethroids, can lead to non-target effects and the development of insecticide resistance (Liu, Xu, Zhu, & Zhang, 2006; Maharaj, 2011). Consequently, there exists an urgent need to develop alternative insecticides to supplement pyrethroids for control of a wide variety of insect-vectored diseases (Maharaj, 2011; Pridgeon, Becnel, Clark, & Linthicum, 2009b; Pridgeon et al., 2008).

An alternative to conventional insecticides is the use of natural products from plants that produce phytochemicals as defense mechanisms against microorganisms and predators. Such chemicals may serve as candidate products for controlling a wide variety of insect vectors. Recent efforts have focused on identification and utilization of plant extracts or phytochemicals as potential sources of commercial mosquito control agents or bioactive chemical compounds (Quinn, Bernier, & Booth, 2007; Yang et al., 2002). Members of the Lamiaceae (mint family), in particular, have been shown to be sources of essential oils having insecticidal and insect repellent properties (Ayvaz, Sagdic, Karaborklu, & Ozturk, 2010; Çalmaşur, Aslan, & Şahin, 2006; Conti, Canale, Cioni, & Flamini, 2010; Odeyemi, Masika, & Afolayan, 2008; Tabanca et al., 2013; Yildirim, Kordali, & Yazici, 2011).

The genus *Caryopteris* Bunge (Lamiaceae) consists of 16 species native to China and East Asia (Abu-Asab, Cantino, Nowicke, & Sang, 1993; Flora of China Editorial Committee, 1994). The composition of essential oils has previously been investigated for several species: *C. forrestii* Diels (Pu, Shi, Yang, Zhang, & Lü, 1984); *C. incana* (Thunberg ex Houttuyn) Miquel (Chu, Liu, Zhou, Du, & Liu, 2011; Kim, 2008; Pu et al., 1984), *C. mongholica* Bunge (Shatar & Adams, 1999), *C. tangutica* Maximowicz (Dai, Zhang, & Liao, 2012; Yan & Wang, 2009), and *C. trichosphaera* W. Smith (Pu et al., 1984). *Caryopteris incana* has been identified as a source of new glycosides (Park et al., 2014) and *C. mongholica* has yielded new alkaloids (Dumaa et al., 2012). Essential oil of *C. incana* has demonstrated strong insecticidal activities against the maize weevil, *Sitophilus zeamais* Mots. (Coleoptera, Dryophthoridae) (Chu et al., 2011).

Caryopteris ×*clandonensis* A. Simmonds ex C. H. Curtis (bluebeard, blue mist shrub, false spirea) is a hybrid between *C. incana* and *C. mongholica*, originating as a chance seedling in the garden of Arthur Simmonds in Surrey, England, in 1933. Since then, additional ornamental cultivars have been selected by horticulturists into the nursery trade (Chicago Botanic Garden, 2014). *C.* ×*clandonensis* is valued in the landscape for its mounded growth habit, showy display of blue flowers in summer, and attractiveness to insect pollinators. *C.* ×*clandonensis* 'Durio' Pink Chablis™, unique among the bluebeards in having pink flowers (Figures 1-3), was discovered as a chance seedling in 1998 by Dalton Durio of Louisiana Nursery, Opelousas, LA, USA [U.S. Plant Patent No. PP16,913 (Durio, 2006)].

Previous research has examined chemical constituents of *C.* ×*clandonensis*. *Caryopteris* ×*clandonensis* was found to be a source of two new keto-glycosides, clandonoside and 8-*O*-acetylclandonoside (Hannedouche, Jacquemond-Collet, Fabre, Stanislas, & Moulis, 1999), the pyranojuglone pigment α-caryopterone (Matsumoto, Mayer, & Eugster, 1969), and quinones with strong molluscicidal activity (Hannedouche, Souchard, Jacquemond-Collet, & Moulis, 2002). Essential oil of *Caryopteris* ×*clandonensis* was found to be less effective than oils from other aromatic plants when tested in vapor phase against foodborne bacteria (Nedorostova, Kloucek, Kokoska, Stolcova, & Pulkrabek, 2009).

In a cooperative effort involving multiple institutions, we are evaluating new plant extracts and pure compounds for mosquito repellent and larvicidal activity as part of the Department of Defense Deployed War-Fighter Protection (DWFP) research program (Cope, Strickman, & White, 2008; Linthicum et al., 2007). The DWFP program emphasizes identification and testing of new classes of chemistry for control of insect vectors and new tools for chemical application suited to the protection of troops and human populations after natural disasters. Taking into account the necessity of developing new mosquito repellents with more favorable environmental properties, the objectives of the current study were to determine the composition of essential oil obtained from the ornamental shrub *Caryopteris* ×*clandonensis* 'Durio' Pink Chablis™ (Lamiaceae) and to examine the repellent and larvicidal activities of the essential oil against the yellow fever mosquito, *Aedes aegypti*.

Materials and Methods

Plant Material and Essential Oil Distillation

Plants of *C. ×clandonensis* 'Durio' Pink Chablis™ (Spring Meadow Nursery Inc., Grand Haven, MI, USA), a vegetatively propagated clone of bluebeard, were used in this study. Plants were grown outdoors in 11.4-L containers in a peatmoss and pine bark-based substrate at the South Mississippi Branch Experiment Station (SMBES) in Poplarville, MS (30°50'26"N, long. 89°32'46"W; USDA hardiness zone 8b). Voucher specimen #9 was deposited at the SMBES for future reference. Aboveground parts were harvested from 9-month-old plants in June 2009 and air-dried for three weeks inside an air-conditioned building (25°C max.). Dried plant material was packed loosely into cardboard boxes to avoid crushing and stored in the same building until shipment to the National Center for Natural Products Research in Oxford, MS for distillation of essential oils. The air-dried aerial parts of *C. ×clandonensis* were subjected to water distillation using a Clevenger-type apparatus to obtain the oil (Figure 4). Light olive-green oil was obtained with a yield of 0.05% (v/w).

Gas Chromatography and Gas Chromatography–Mass Spectrometry Analysis of Essential Oil

The essential oil was analyzed by gas chromatography (GC) with a flame ionization detector (FID) and gas chromatography–mass spectrometry (GC-MS) using an Agilent 5975 GC-mass selective detector (MSD) system. For the GC-MSD analysis, an Innowax fused silica capillary (FSC) column (60 m × 0.25 mm, 0.25 µm film thickness) was used with helium as the carrier gas (0.8 mL/min). The oven temperature was kept at 60 °C for 10 min, then programmed to 220 °C at a rate of 4 °C/min, then maintained constant at 220 °C for 10 min, and finally programmed to 240 °C at a rate of 1 °C/min. The injector temperature was set at 250 °C. The split flow was adjusted at 50:1. Mass spectra were recorded at 70 eV with the mass range m/z 35 to 450. The GC analysis was performed using an Agilent 6890N GC system. FID detector temperature was set to 300 °C and the same operational conditions were applied to a duplicate of the same column used in GC-MS analysis. Simultaneous auto injection was done to obtain equivalent retention times. Relative percentages of the separated compounds (Table 1) were calculated from integration of the peak areas in the GC-FID chromatogram.

Individual components were identified by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes (Curvers , Rijks, Cramers, Knauss, & Larson, 1985) and by computer matching with commercial mass spectral libraries (Wiley GC/MS Library, MassFinder 3 Library) and in-house “Baser Library of Essential Oil Constituents” built up from the authentic samples, known oils, and mass literature data (ESO, 2000; Joulain & König, 1998; König, Joulain, & Hochmuth, 2004; McLafferty and Stauffer, 1989).

Mosquito Bioassays

Mosquitoes

Aedes aegypti (1952 Orlando strain) larvae and adults used in these studies were from a laboratory colony maintained at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural, and Veterinary Entomology, USDA-ARS, Gainesville, FL, USA. For larval bioassays, the eggs were hatched and the larvae were maintained at an ambient temperature of 78 ± 3 °C.

Mosquito repellent assay (Cloth patch assay)

Repellency was determined as the Minimum Effective Dosage (MED), which is the minimum threshold surface concentration necessary to prevent mosquitoes from biting through the treated surface (Schreck, Posey, & Smith, 1977) Approximately 500 (± 10%) mosquitoes were collected and loaded into a test cage

(size of 45 cm x 37.5 cm x 35 cm) and held in the cage for 25 (± 2.5) min before initiating repellency assays. Serial dilutions were then made such that the concentrations on the cloth for the remaining 1 mL solution were: 0.375, 0.094, 0.047, 0.023, and 0.011 mg/cm^2. Each concentration was tested to determine the point where the repellent failed for each of the volunteers in the study; this concentration was averaged and reported. Each test was conducted by having a volunteer affix the treated cloth onto a plastic sleeve to cover a 32 cm^2 window previously cut into the sleeve. Each of the volunteers wore this sleeve/cloth assembly above a nylon stocking covering their arm, with their hands protected by a glove (Katritzky et al., 2010). The arm with the sleeve/cloth assembly was inserted into a cage, where approximately 500 female *Ae. aegypti* mosquitoes (aged 6-10 days) had been preselected as host-seeking using a draw box (Posey & Schreck, 1981). Failure of the repellent treatment is 1% bite through, *i.e*. the volunteer receives 5 bites through the cloth over the sleeve window in the 1 minute assay. There were three human volunteers in this study and all three provided written informed consent to participate in this study as part of a protocol (636-2005) approved by the University of Florida Human Use Institutional Review Board (IRB-01).

Mosquito larvicidal assay

Bioassays were conducted using the system described by Pridgeon et al. (2009a) to determine the larvicidal activity of the essential oils against *Ae. aegypti*. Five 1-d-old larvae were transferred to individual wells of a 24-well tissue culture plates in a 30-40 µL droplet of water. Fifty µL of larval diet of 2% slurry of 3:2 beef liver powder (Now Foods, Bloomingdale, Illinois) and Brewer's yeast (Lewis Laboratories Ltd., Westport, CT) and 1 mL of deionized water were added to each well by using a Finnpipette® stepper pipetter (Thermo Fisher, Vantaa, Finland). *C. ×clandonensis* 'Durio' essential oil was diluted in DMSO. Eleven microliters of the test chemical was added to the wells, while 11 µL of DMSO was added to the control treatments. After treatment application, the plates were swirled in clockwise and counterclockwise motions and front to back and side to side five times to ensure even mixing of the tested compounds. Permethrin (46.1% *cis* – 53.2% *trans*; Chemical Service, West Chester, PA) at 0.025 ppm was used as positive control. Larval mortality was recorded 24 h post treatment.

Results and Discussion

A total of 50 compounds were identified in the essential oil of *C. ×clandonensis* 'Durio' Pink Chablis™ (Table 1). The main components were characterized as α-copaene (8.3%), limonene (7.2%) and δ-cadinene (6.3%), followed by *trans-p*-mentha-2,8-dien-1-ol (4.6%), *trans-p*-mentha-1(7),8-dien-2-ol (4.5%), *cis-p*-mentha-2,8-dien-1-ol (4.0%), and hotrienol (3.8%). Among the main compounds in essential oil of *C. ×clandonensis* 'Durio', limonene and δ-cadinene have also been previously reported as major compounds in essential oil of *C. mongholica* from Mongolia (Shatar & Adams, 1999), with limonene also reported as a major compound in essential oil of *C. incana* from Jiangxi, China (Sun, Ye, & Chen, 2004). Otherwise, main components in *C. ×clandonensis* 'Durio' essential oil mostly differed from those previously reported for *C. incana* and *C. mongholica*, the parent species of *C. ×clandonensis*. Shatar & Adams (1999) reported main constituents of essential oil from leaves and flowers of *C. mongholica* from Mongolia were α-thujene (18.7%); (*E*)-β-ocimene (11.0%); limonene (8.8%); β-pinene (8.0%) terpinene-4-ol (7.2%); α-pinene (6.3%); sabinene (5.6%); sylvestrene (2.4%); γ-terpinene (2.3%); germacrene-D (2.3%) and δ-cadinene (2.1%). Composition of essential oils from aerial parts of *C. incana* from China and Korea differed by source of the plant material (Chu et al., 2011; Sun, Ye, & Chen, 2004; Pu et al., 1984; Kim, 2008). Main components produced by plants were estragole (24.8%), linalool (14.0%), 1,8-cineol (5.2%), and δ-guaiene (4.1%) using plants from Guangdong, China (Chu et al., 2011).; linalool (16.3%), perillalcohol (15.3%), carvone (14.7%), and orthodene (9.7%) using plants from Jiangxi, China (Sun, Ye, & Chen, 2004); limonene (38.5%), α-terpenene (17.3%), β-pinene (12.9%) and *ρ*-

cymene (12.6%) using plants from Sichuan, China (Pu et al., 1984); and 4,6,6-tri-methyl [1S-(1α,2β,5α)]-bicyclo[3.1.1]hept-3-en-2-ol (11.8%), τ-cadinol (9.4%), myrtenyl acetate (9.2%), pinocarvone (7.0%), 1-hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene (6.3%), and δ-3-carene (6.2%) using plants from Korea (Kim, 2008).

The mosquito repellent assay using *Ae. aegypti* mosquitoes revealed the essential oil of *C. ×clandonensis* 'Durio' to have a MED for repellency of 0.250 ± 0.109 mg/cm^2; however, this indicated a mild ability to repel *Ae. aegypti* compared with the reference standard, DEET (MED=0.039 ± 0.014 mg/cm^2). In the mosquito larvicidal screening assay, *C. ×clandonensis* 'Durio' essential oil gave 90%, 20% and 0% mortality of the 1-d-old *Ae. aegypti* larvae at the concentrations of 125, 62.5 and 31.25 ppm, respectively.

This study provides the first report on the composition of the essential oil of the interspecific ornamental *C. ×clandonensis* 'Durio' Pink Chablis™ and its assessment as a mosquito repellent and larvicide. Although the essential oil exhibited mild repellency and weak larvicidal activity against *Ae. aegypti*, further investigation for unique chemical constituents may be warranted based on previous findings with C. ×*clandonensis* (Hannedouche et al., 1999; Hannedouche et al., 2002) and other species of *Caryopteris* (including *C. mongholica*, a parent of *C. ×clandonensis*) (Dai et al., 2012; Dumaa et al., 2012; Park et al., 2014). Being a vegetatively propagated clone, chemical constituents of *C. ×clandonensis* 'Durio' are likely to remain more consistent from one harvest to another than would wild-collected forms of *Caryopteris*.

Table 1. Composition of the essential oil of *Caryopteris ×clandonensis* 'Durio' Pink Chablis™.

RRI	**Compound**	**%**	**Identification method**
1032	α-Pinene	0.3	t_R, MS
1076	Camphene	0.1	t_R, MS
1118	β-Pinene	0.4	t_R, MS
1203	Limonene	7.2	t_R, MS
1220	*cis*-Anhydrolinalool oxide	0.5	MS
1224	*o*-Mentha-1(7),5,8-triene	2.5	MS
1253	*trans*-Anhydrolinalool oxide	0.4	MS
1261	menthatriene isomer*	6.6	MS
1280	*p*-Cymene	0.3	t_R, MS
1319	Dihydrotagetone	0.1	MS
1408	1,3,8-*p*-Menthatriene	0.3	MS
1452	α,*p*-Dimethylstyrene	2.4	MS
1452	1-Octen-3-ol	0.9	MS
1478	*cis*-Linalool oxide	0.2	MS
1492	Cyclosativene	0.5	MS
1497	α-Copaene	8.3	t_R, MS
1553	Linalool	2.7	t_R, MS
1612	β-Caryophyllene	0.5	t_R, MS
1616	Hotrienol	3.8	MS
1639	*trans-p*-Mentha-2,8-dien-1-ol	4.6	MS
1661	Alloaromadendrene	0.3	MS
1678	*cis-p*-Mentha-2,8-dien-1-ol	4.0	MS
1700	*p*-Mentha-1,8-dien-4-ol	0.1	MS
1704	Myrtenyl acetate	0.4	MS
1706	α-Terpineol	0.4	t_R, MS
1708	Ledene	0.6	MS
1740	α -Muurolene	0.3	MS
1751	Carvone	2.7	t_R, MS
1773	δ-Cadinene	6.3	MS

1797	*p*-Methyl acetophenone	0.2	MS
1807	Perilla aldehyde	0.2	t_R, MS
1811	*trans-p*-Mentha-1(7),8-dien-2-ol	4.5	MS
1845	*trans*-Carveol	2.3	t_R, MS
1849	Calamenene	1.0	MS
1864	*p*-Cymen-8-ol	0.4	t_R, MS
1896	*cis-p*-Mentha-1(7),8-diene-2-ol	2.0	MS
1941	α-Calacorene	3.1	MS
1984	γ-Calacorene	0.9	MS
2008	Caryophyllene oxide	0.4	t_R, MS
2057	Ledol	1.2	MS
2080	Cubenol	0.2	MS
2088	1-*epi*-Cubenol	0.3	MS
2089	6-Methyl-5(3-methylphenyl)-2-heptanone	0.5	MS
2104	Viridiflorol	0.5	MS
2161	Muurola-4,10(14)-dien-1-ol	1.3	MS
2198	Thymol	2.7	t_R, MS
2239	Carvacrol	0.6	t_R, MS
2256	Cadalene	2.2	MS
2289	Oxo-α-Ylangene	2.0	MS
2411	4-Isopropyl-6-methyl-1-tetralone	0.4	MS
	Total	84.6	

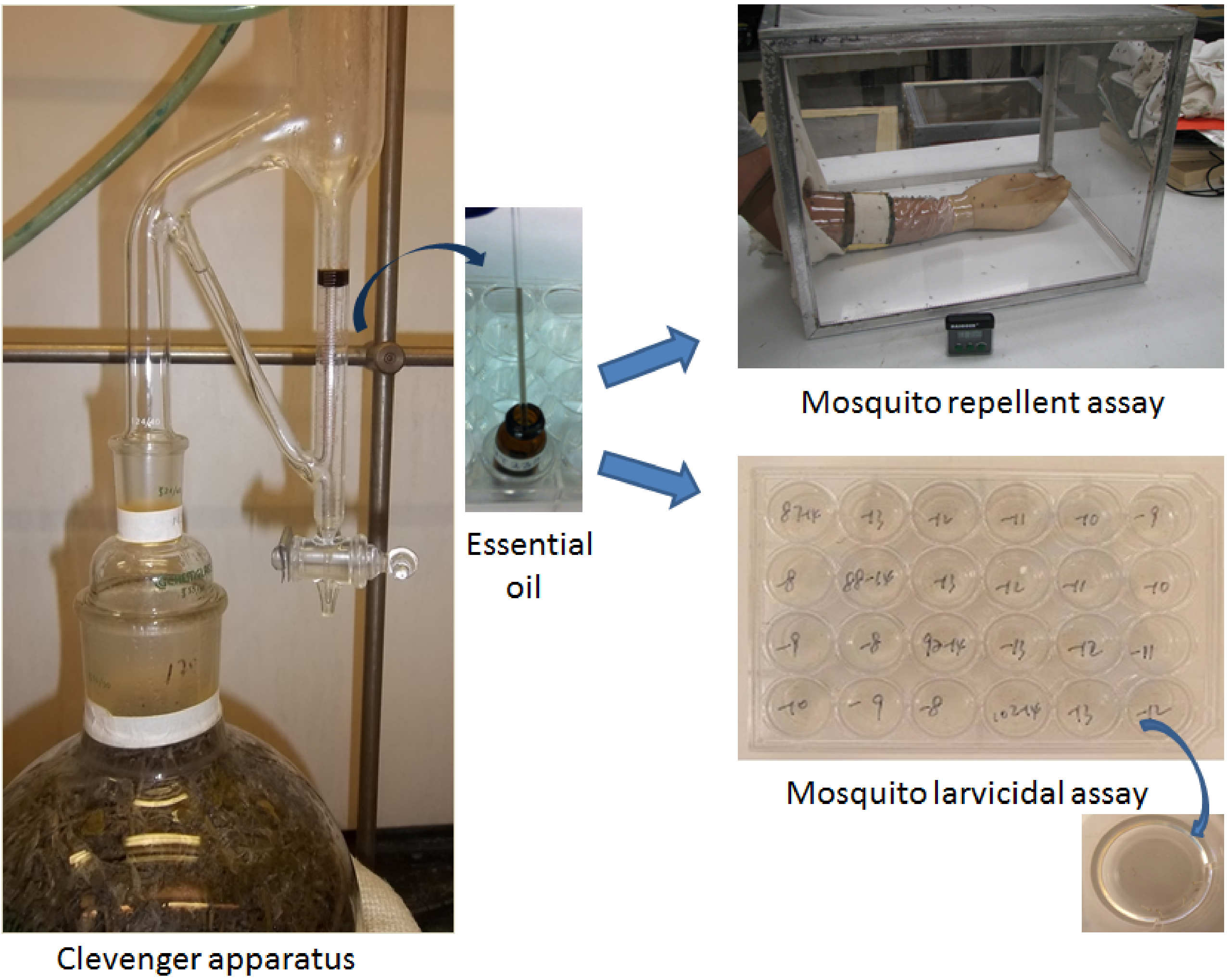
*: Correct isomer could not identified; RRI;Relative retention indices calculated against *n*-alkanes; % calculated from FID data; Identification method, t_R, identification based on the retention times of genuine compounds on the HP Innowax column; MS**,** identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

Figure 1. *C. ×clandonensis* 'Durio' Pink Chablis™ growing in a landscape setting. (Photo by Spring Meadow Nursery, Inc.)

Figure 2. Inflorescences of *C.* ×*clandonensis* 'Durio' Pink Chablis™**.** (Photo by Spring Meadow Nursery, Inc.)

Figure 3. Close-up of the flowers of *C.* ×*clandonensis* 'Durio' Pink Chablis™. (Photo by Spring Meadow Nursery, Inc.)

Figure 4. Essential oil of *Caryopteris ×clandonensis* 'Durio' Pink Chablis™ was obtained from aerial parts by water distillation using a Clevenger-type apparatus and the oil was subjected to mosquito repellent and larvicidal bioassays.



ACKNOWLEDGMENTS

This study was supported in part by USDA/ARS grant No. 56-6402-1-612, Deployed War-Fighter Protection Research Program Grant funded by the U.S. Department of Defense through the Armed Forces Pest Management Board, and a Special Research Initiative grant from the Mississippi Agricultural and Forestry Experiment Station. We thank Nathan Newlon, Greg Allen, Dr. Maia Tsikolia, and Dr. James J. Becnel, USDA-ARS, Gainesville, FL for supplying mosquito eggs. We also thank Cecil Pounders, Eric Stafne, and Stephen Stringer for reviewing an early draft of the manuscript. This paper was approved for publication as Journal Article No. J-00000 of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University.

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